0026-895X/93/030649-08$03.00/0

MOLECULAR PHARMACOLOGY, 44:649-656

Transfected D_2 Short Dopamine Receptors Inhibit Voltage-Dependent Potassium Current in Neuroblastoma × Glioma Hybrid (NG108–15) Cells

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Received November 24, 1992; Accepted June 26, 1993

SUMMARY

Two isoforms of the D_2 dopamine receptor exist, termed D_2 short (D_{2S}) and D_2 long, which differ by the presence or absence of 29 amino acids. To examine the possible coupling of the D_{2S} isoform to voltage-dependent K^+ current, NG108–15 cells that were transfected with and stably express this isoform were studied using whole-cell patch-clamp techniques. In transfected, but not untransfected, cells dopamine and quinpirole (QUIN) reduced the normally observed peak outward K^+ current, and this effect was abolished by the D_2 antagonist sulpiride but not by the α_2-adrenergic receptor antagonist idazoxan or the D_1 antagonist (*R*)-(+)-SCH-23380. The D_1 receptor agonist SKF 38393 had no effect. QUIN-induced inhibition of K^+ current was prevented by loading the cells with the Ca^{2+}-chelator 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, suggesting a critical role for intracellular Ca^{2+} mobilization. In contrast, reduction of the concentration of extracellular Ca^{2+} and inclusion of the Ca^{2+} channel blocker cobalt did not modify the reduction of K^+ current produced by stimulation of D_{2S} receptors. A critical role for intracellular calcium mobilization in the observed effects was further supported by the observation that increases in cytosolic Ca^{2+} produced by thapsigargin mimicked the effect of QUIN, whereas intracellular ryanodine, which blocks Ca^{2+} mobilization, abolished the QUIN responsiveness. Finally, the effect of D_{2S} activation on K^+ current was not modified by pretreatment of the cells with pertussis toxin. These results suggest that the D_{2S} dopamine receptor expressed in NG108–15 cells inhibits the activity of native K^+ current via a mechanism that is dependent upon the mobilization of intracellular Ca^{2+} and does not involve a pertussis toxin-sensitive G protein.

Dopaminergic neurotransmission has been studied extensively because of the hypothesized involvement of this neurotransmitter in a wide variety of psychiatric and neurological disorders. DA receptors have been classified into two distinct subtypes, D_1 and D_2, based on their biochemical and pharmacological characteristics (1, 2). It is now clear that the activation of D_2 receptors modifies adenylyl cyclase activity, phosphoinositide hydrolysis, intracellular calcium, several types of potassium and calcium channels (see Ref. 3), and arachidonic acid release (4–6). Recently, we showed that the D_2 receptors present upon DA neurons modulate the activity of at least two different K^+ currents, and this effect involves the activated α subunit of the G protein G_o (7).[1]

Over the last few years, characterization of the D_2 receptor gene has revealed that two molecular forms of the receptor are produced via alternative mRNA splicing (8–10). These two receptor isoforms, called D_{2S} and D_{2L}, differ by a 29-amino acid sequence located in the third cytoplasmic loop. Because this loop has been proposed to interact with G proteins in this class of receptors, it has been suggested that the two forms of the D_2 receptor might couple to different intracellular pathways (9, 11–13). Because both receptor subtypes are typically expressed in the same tissues (14–16) and display the same pharmacological characteristics (11, 12), it is very difficult to study their functions separately in cells that endogenously express them. Expression of cloned receptors in cell lines allows investigation of cellular mechanisms linked to isolated receptor subtypes. To study cellular mechanisms coupled to the D_{2S} receptor isoform, neuroblastoma × glioma hybrid cells (NG108–15) were transfected to stably express this DA receptor subtype. The establishment of this transfected cell line made it feasible to begin

[1] L.-X. Liu, R. Y. Shen, G. Kapatos, and L. A. Chiodo. Dopamine neuron membrane physiology: characterization of the transient outward current, (I_A) and demonstration of a common signal transduction pathway fro I_A and I_K. Submitted for publication.

This work was supported in part by United States Public Health Grants MH-41557 (L.A.C) and NS-26081 (G.K.).

ABBREVIATIONS: DA, dopamine; TEA, tetraethylammonium; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GDPβS, guanosine 5′-*O*-(2-thio)diphosphate; D_{2S}, D_2 short; D_{2L}, D_2 long; QUIN, quinpirole; DADLE, D-Ala^2-D-Leu^5-enkephalin.

to examine the possible coupling of these receptors to K^+ currents and the signal transduction pathways involved.

Materials and Methods

NG108–15 cell culture and transfection. NG108–15 cells were routinely cultured in high-glucose Dulbecco's modified essential medium with L-glutamine (GIBCO), containing 1 mM sodium pyruvate, 10% fetal bovine serum, hypoxanthine-aminopterin-thymidine medium supplement (Sigma), and 30 μg/ml gentamycin. Cells seeded into 150- × 20-mm plates were transfected with 60 μg of pSRα-D_{2S} plasmid DNA plus 6 μg of pMam-Neo (Invitrogen), using the $CaPO_4$ precipitation method. The 2.4-kilobase cDNA encoding the D_{2S} receptor was isolated from a rat striatal library and used in the construction of the expression plasmid vector. This construct has been previously used to express D_{2S} receptors in Chinese hamster ovary cells (17). Cells were subcultured after 48 hr, followed by selection for stable transfectants through the addition of 300 μg/ml G418 (GIBCO) to the medium. Individual cell colonies were isolated after 1 week of selection and screened for D_2 receptor binding as described (9). Receptor-expressing cell lines were subsequently subcloned by limiting dilution, to generate clonal cell lines. The cell line used in this study was found to express 80 fmol/mg of protein of specific D_2 receptor binding activity.

Electrophysiological methods. Coverslips with attached NG108–15 cells were transferred from 24-well plates into 35-mm culture dishes containing the basic external recording solution (in mM: NaCl, 135; KCl, 5.4; $CaCl_2$, 1.8; $MgSO_4$, 0.8; glucose, 20; HEPES, 5; plus 2 μM tetrodotoxin, adjusted to pH 7.3 with Tris base). In some experiments the effects of external Ca^{2+} were studied by adding $CoCl_2$ (1 mM) to the external medium and reducing the $CaCl_2$ concentration to 0.8 mM. The dish was placed on the stage of an inverted microscope (Axiovert 35; Ziess) and visualized using Hoffman differential interference contrast optics at ×400.

Whole-cell patch-clamp recordings were performed at room temperature (20–24°) in a static bath chamber. Patch pipettes were pulled from micro-hematocrit capillary tubes (Drummond Scientific) using a two-stage process, as described by Hamill *et al.* (18). Pipettes were then fire-polished. The patch electrode tip ranged between 1 and 3 μm and had resistances between 2 and 4 MΩ when filled with normal patch pipette solution. The normal patch electrode solution consisted of (in mM) KCl, 140; $MgCl_2$, 2; $CaCl_2$, 1; HEPES, 10; ATP, 2; cAMP, 0.25; and BAPTA, 0.5; adjusted to pH 7.3 with KOH. In experiments designed to address the role of intracellular Ca^{2+}, the concentration of the Ca^{2+}-chelator BAPTA within the patch pipette was modified. In other experiments, 20 μM or 10 μM ryanodine was included in the external solution or pipette solution, respectively, to block Ca^{2+} release from internal stores (19–22). Ryanodine stock solution (50 mM) was made in 95% ethanol. Experiments were carried out with the same final ethanol concentration, to control for the effect of ethanol. No effects of this vehicle were observed. When TEA (30 mM) was added to the bathing medium, an equimolar concentration of KCl was removed. In some experiments GDPβS was included in the patch recording solution at a concentration of 100 μM. All solutions were filtered through a 0.45-μm membrane filter. Recordings were made using an Axopatch 200 amplifier (Axon Instruments, Burlingame, CA). The signal was low-pass filtered at 2 kHz and digitized using pCLAMP hardware and software (Axon Instruments). Data were stored on floppy disks for later off-line analysis.

After successful establishment of a whole-cell recording, the fast and whole-cell capacitances and series resistance were compensated. To activate steady state outward currents, the cell was voltage clamped at a holding potential of −50 mV and stepped to membrane potentials between −90 and +70 mV for a duration of 300 msec (presentation rate, 0.2 Hz). This operation was repeated to ensure that the amplitude of the current was stable. Only cells that displayed stable current amplitudes were used. All drugs applied to the outside of the cell were dissolved in the external solution and were applied via a 10–15-μm-diameter pressure ejection pipette. The drug application protocol consisted of 5–10 psi (200-msec duration) pulses delivered 200 msec before every voltage command by a Picospritzer II (General Valve Corp., Fairfield, NJ). In other experiments, a perfusion system was used to allow for the examination of the effects of different steady state concentrations of the D_2 receptor agonist QUIN on the outward currents observed.

Where appropriate, data are presented as mean ± standard error of percentage of current reduction recorded in the last test pulse (+70 mV) before and after drug application. Statistical significance ($p < 0.05$) was evaluated by analysis of variance and t test.

Results

All cells studied ($n = 183$) displayed a prominent K^+-dependent outward current. This outward current was activated at membrane potentials positive to −20 from a holding potential of −50 mV. The outward current observed in transfected NG108–15 cells was indistinguishable from that seen in native (untransfected) cells ($n = 35$) (Fig. 1). The peak outward current amplitude was usually between 1500 and 3000 pA (at +70 mV membrane potential) but ranged from 727 to 5670 pA. The variability in peak outward current was not related to whether or not the cells were transfected but rather appeared to be correlated with differences in cell size. This issue was not systematically studied, but the peak magnitude of the current under control conditions had no effect on the responsiveness of transfected cells to D_2 receptor activation. The observed current was reduced by 85–95% when 30 mM TEA was added to the bathing solution ($n = 7$) (Fig. 2).

DA and QUIN inhibit K^+ current. Pressure application of DA (100 μM) reduced the amplitude of the outward current studied in transfected cells by 22.8 ± 3.4% ($n = 8$) (Fig. 3A), while having no effect on the current observed in untransfected

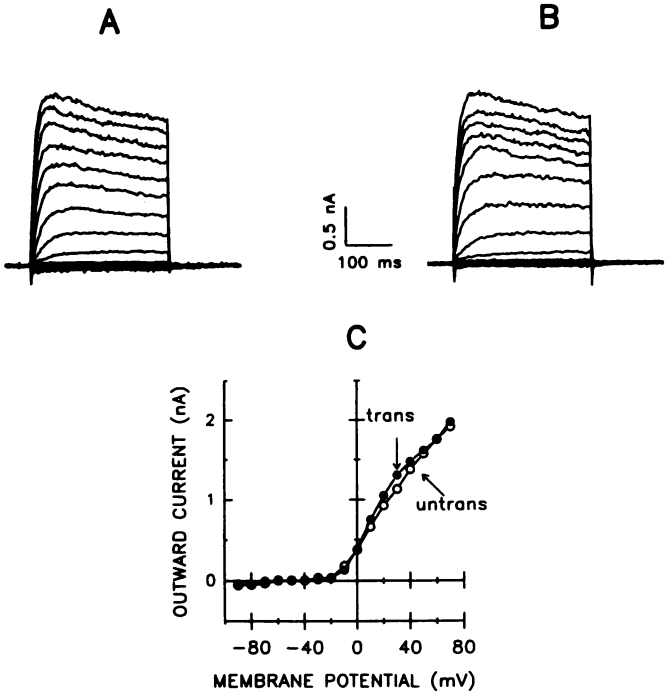

Fig. 1. Current-voltage relationship of outward K^+ current observed in transfected (*trans*) and untransfected (*untrans*) NG108–15 cells. A and B, Whole-cell current typically observed in untransfected (A) and transfected (B) cells. It can be seen that the currents studied are essentially identical under these two conditions. C, Current-voltage relationship from A and B. The current measured at the end of the voltage step is plotted relative to the membrane potential. The holding potential for both cells was −50 mV.

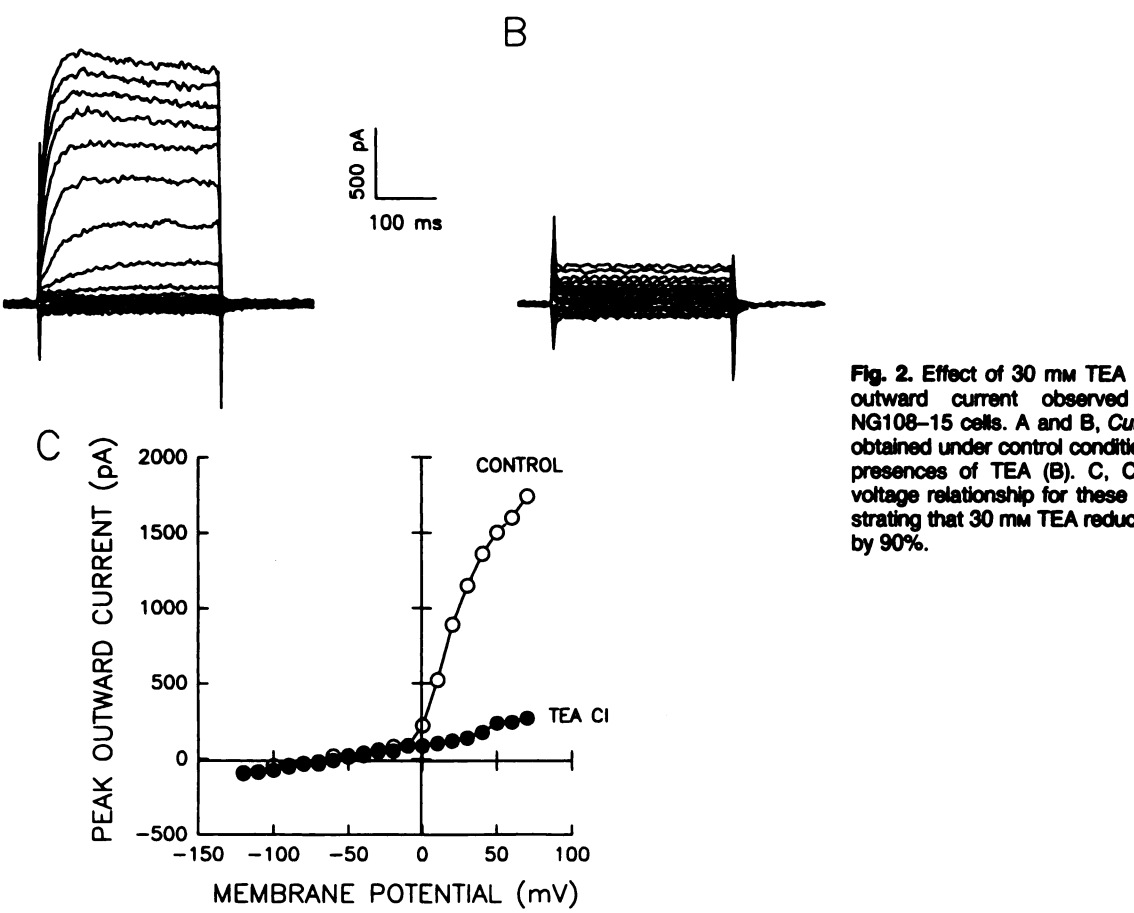

Fig. 2. Effect of 30 mM TEA on the whole-cell outward current observed in transfected NG108–15 cells. A and B, *Current traces* were obtained under control conditions (A) and in the presences of TEA (B). C, Complete current-voltage relationship for these currents, demonstrating that 30 mM TEA reduced the K^+ current by 90%.

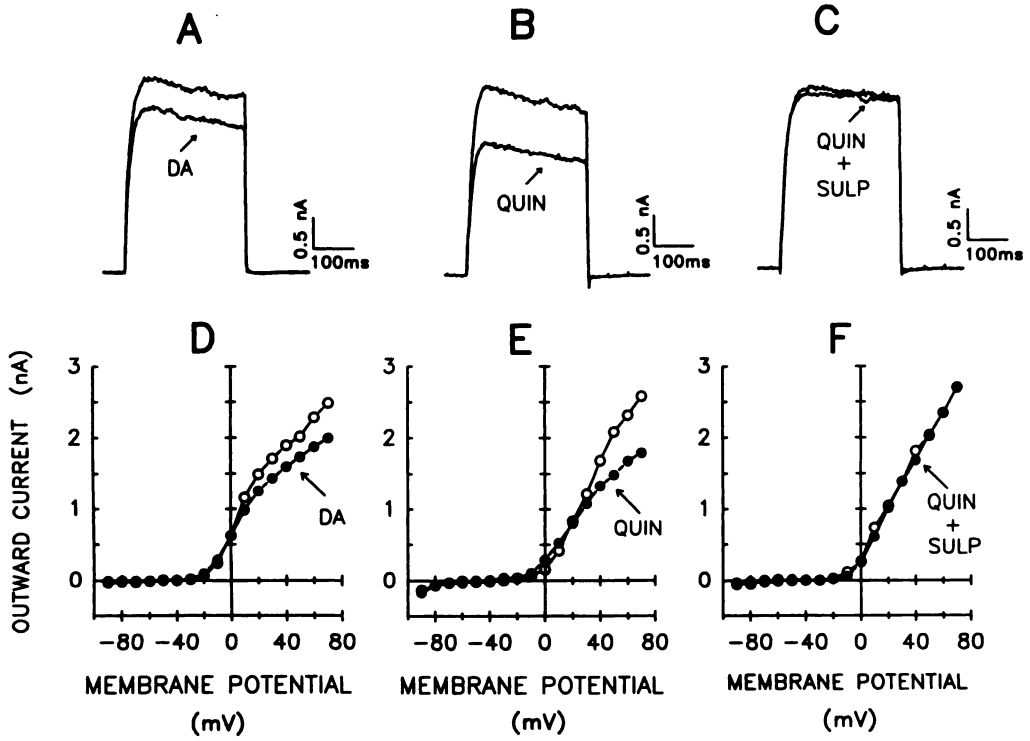

Fig. 3. Effect of D_{2S} receptor stimulation on the whole-cell outward current observed in transfected NG108–15 cells. A and B, The pressure application of 100 μM DA (A) or 100 μM QUIN (B) significantly reduced the outward current observed. C, The effect of QUIN (100 μM) application was completely blocked by the D_2 receptor antagonist sulpiride (*SULP*) (40 μM). The outward current was evoked by stepping the membrane to a potential of +70 mV from a holding potential of −50 mV (A-C were derived from three different cells). D, E, and F, complete current-voltage relationship of the cells illustrated in A-C.

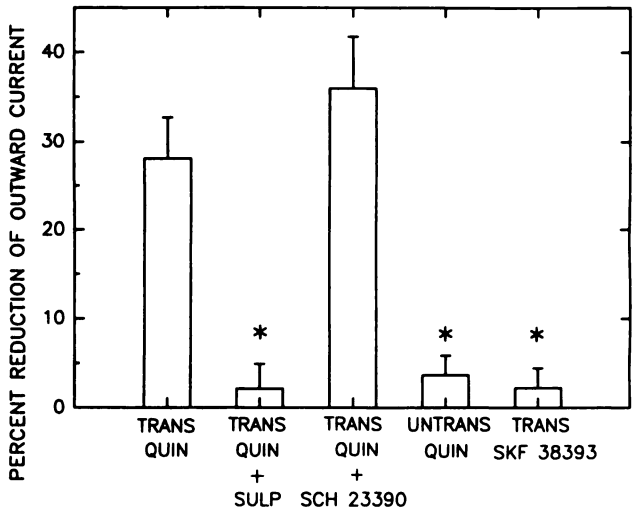

Fig. 4. The effect of QUIN on steady state K^+ current is due to the activation of D_2 receptors. *Bar graph*, effects of the various pharmacological treatments on the percentage reduction of K^+ current in transfected NG108–15 cells. QUIN (100 μM) reduced the outward current observed in transfected cells by approximately 28% (*TRANS QUIN*) (n = 13). Coadministration of the D_2 receptor antagonist sulpiride, at a concentration of 40 μM, essentially abolished the inhibition typically produced by QUIN (*TRANS QUIN + SULP*) (n = 7). In marked contrast, the coapplication of the D_1 receptor antagonist SCH-23390, at a concentration of 40 μM, did not alter the effectiveness of QUIN (*TRANS QUIN + SCH 23390*) (n = 7). QUIN (100 μM) had no effect in untransfected NG108–15 cells (*UNTRANS QUIN*) (n = 7). Similarly, the D_1 receptor agonist SKF 38393 (100 μM) did not significantly alter the outward current observed in transfected cells (*TRANS SKF 38393*) (n = 6) All data represent the mean ± standard error of the percentage reduction of observed K^+ current. The percentages of reduction were calculated at the end of the voltage-clamp step to a membrane potential of 70 mV, before and after the drug application (holding potential, −50 mV). *, $p <$ 0.002, compared with QUIN-induced reduction of outward current in transfected cells.

cells (n = 12) (data not shown). Because DA is not specific for D_2 receptors, we also examined the effect of the D_2-selective agonist QUIN. QUIN also reduced the steady state outward current observed in transfected cells (Fig. 3B) and this effect was dose dependent, whereas no effect was observed in untransfected NG108–15 cells (Fig. 4). This effect was reversible, with the current returning to 90% of predrug levels within 20 min, and control application of external solution had no effect on the outward current (n = 9). In an additional group of cells, QUIN (100 μM)-induced inhibition of K^+ current was abolished when the agonist was coapplied with 40 μM concentrations of the D_2 antagonist (−)-sulpiride (n = 7) (Figs. 3C and 4) and no effect was observed when the D_1-selective agonist SKF 38393 (100 μM) was applied (n = 6). Moreover, the inhibitory effect of 100 μM QUIN was not significantly modified when QUIN was applied with the α_2-adrenergic receptor antagonist idazoxan (20 μM) (n = 5) or the D_1 antagonist (R)-(+)-SCH-23380 (40 μM) (n = 7). The inhibition of K^+ current was observed at concentrations of QUIN as low as 25 μM (in the pipette), and the maximum reduction of outward current was observed when a concentration of 100 μM was applied from the drug pipette (Fig. 5A). The effective concentration of drug at the receptor cannot be determined, but the distance of the pressure ejection electrode from the cell and the static nature of the bath indicate that it is probably significantly lower. To address this directly, the effects of steady state concentrations of QUIN on the observed K^+ current were studied. As seen in (Fig. 5B), under these conditions QUIN again dose-dependently inhibited the outward current studied, with a concentration of 50 μM producing a 68% reduction of current magnitude. The calculated IC_{50} was 6.6 μM, which is in close agreement with previous observations on cloned D_2 receptors (23). Thus, it appears that the pressure application of 100 μM QUIN produced an inhibition of current that corresponded to a steady state concentration of 2 μM.

Intracellular Ca^{2+} mobilization is involved in the D_{2S} effect. The reduction of outward current induced by QUIN (100 μM) was prevented by loading NG108–15 cells with the Ca^{2+}-chelator BAPTA. When 0.1 mM or 0.5 mM BAPTA was included in the patch pipette solution, QUIN (100 μM) produced 32.5 ± 7.3% and 28.1 ± 4.7% outward current reduction, respectively. In contrast, when 1 or 2 mM was used, this effect was abolished (Fig. 6).

To further study the role of Ca^{2+} in the D_{2S} response, cobalt, thapsigargin, and ryanodine were used in a series of experi-

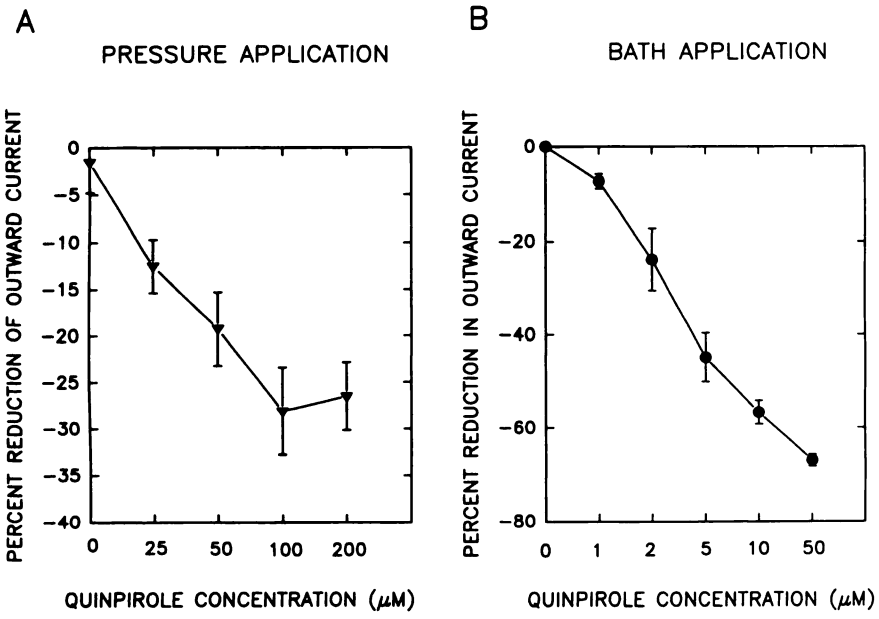

Fig. 5. Dose-dependent inhibition of K^+ current by the D_2 receptor agonist QUIN. A, Dose-response curve for the percentage reduction of outward K^+ current induced by the pressure application of QUIN. B, Dose-response curve for the percentage reduction of outward K^+ current induced by different steady state bath concentrations of QUIN. The calculated IC_{50} was 6.6 μM.

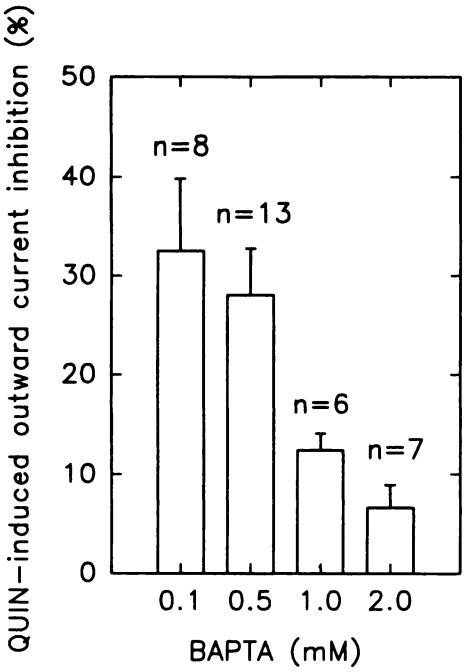

Fig. 6. QUIN-induced inhibition of K^+ current is Ca^{2+} dependent. *Bar graph*, effects of increasing concentrations of BAPTA in the patch pipette solution on the QUIN-induced inhibition of outward current. The data represent the mean ± standard error of the percentage reduction of outward current observed when the membrane was stepped to a potential of 70 mV (holding potential, −50 mV).

ments to selectively manipulate Ca^{2+} entry or intracellular Ca^{2+} release. When 1 mM $CoCl_2$ was present in the bath solution and the concentration of $CaCl_2$ was reduced (from 1.8 to 0.8 mM), no change in the magnitude of the 100 μM QUIN-induced inhibition of outward current was observed ($n = 8$). This result suggests that Ca^{2+} entry during membrane depolarization was not critically involved in the D_{2S}-mediated inhibition of outward current seen in transfected cells. Thus, the effect of BAPTA on D_{2S} response was hypothesized to be due to a reduction of Ca^{2+} released from intracellular stores. To test this possibility, the external application of thapsigargin was employed. Pressure application of thapsigargin reduced the amplitude of the outward current. Thapsigargin at 10 μM and 50 μM reduced the amplitude of the K^+ current by 17.5 ± 3.4% ($n = 9$) and 41.1 ± 6.1% ($n = 8$), respectively (Fig. 7). Thus, in response to thapsigargin, an increase of intracellular Ca^{2+} reduced the outward current in a manner similar to that observed with either DA or QUIN. Moreover, when ryanodine, which prevents Ca^{2+} release from internal stores (19–22), was included in the bath (20 μM) or patch electrode (10 μM), the QUIN inhibition of outward current was dramatically reduced (Fig. 8).

D_{2S} effects are not mediated by a pertussis toxin-sensitive G protein. The D_{2S} receptor response was not mediated by G proteins sensitive to pertussis toxin. Pretreatment with 1 μg/ml pertussis toxin for 4–5 hr did not modify the effect of 100 μM QUIN on K^+ conductance ($n = 8$) (Fig. 9A). To ensure that our pertussis toxin treatment regimen was capable of inhibiting G protein function by ADP-ribosylation, the effect of the opioid DADLE was examined. In NG108–15 cells, DADLE decreases the peak voltage-dependent T-type Ca^{2+} current via a pertussis toxin-sensitive G protein (24). As expected, the inhibition of T current induced by DADLE (2 μM) in control cells ($n = 7$) was prevented by pertussis toxin pretreatment ($n = 9$) (Fig. 9B). It was also observed that this current could be blocked by the inclusion of cobalt in the external bathing medium ($n = 7$) (data not shown). To dem-

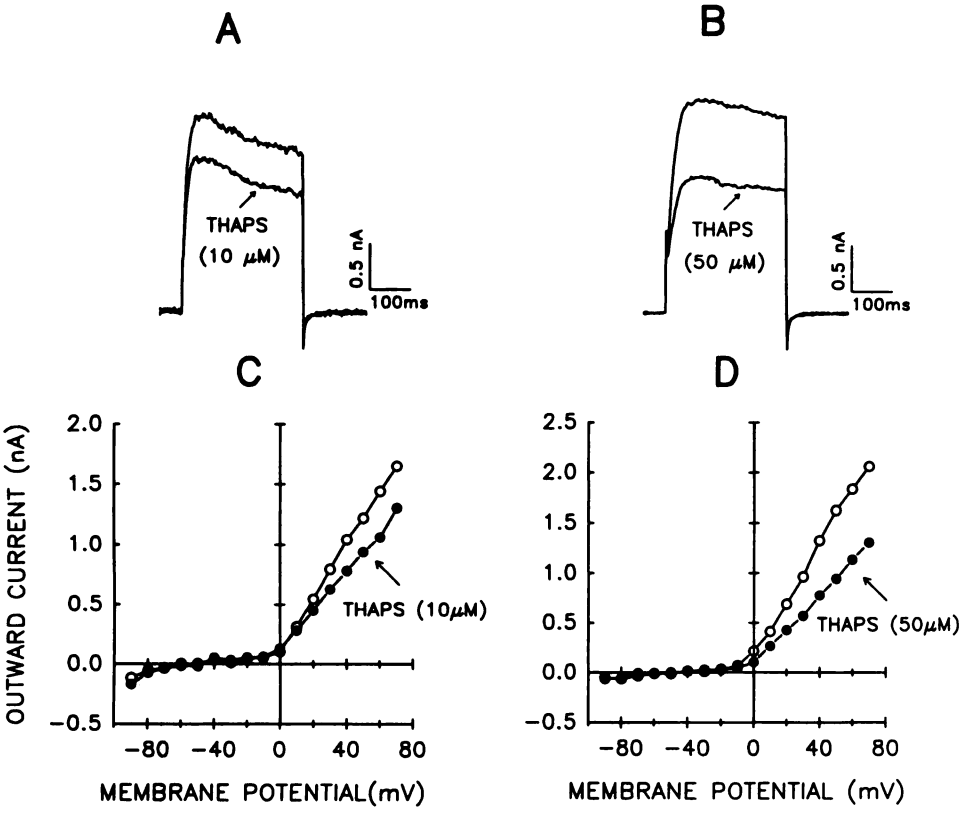

Fig. 7. Effects of thapsigargin-induced increases in intracellular Ca^{2+} on outward currents in transfected NG108–15 cells. Thapsigargin (*THAPS*) was applied by pressure ejection from a micropipette, at two different concentrations. A and B, Typical *current traces* observed before and after the application of either 10 μM or 50 μM thapsigargin (test membrane potential, +70 mV: holding potential, −50 mV). C and D, Overall current-voltage relationships, before (○) and after (●) thapsigargin application, for the cells illustrated in A and B, respectively.

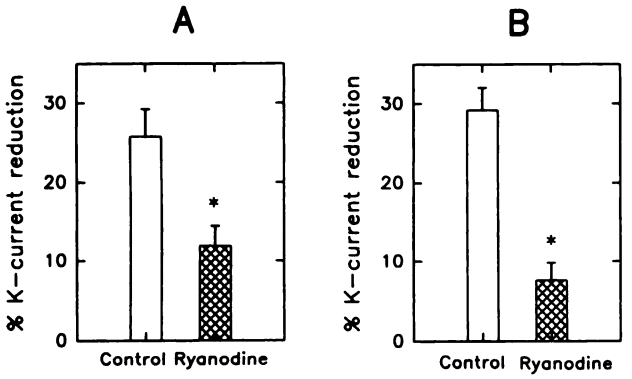

Fig. 8. Effects of ryanodine-induced blockade of intracellular Ca^{2+} mobilization on the QUIN-induced inhibition of outward current. *Bar graphs*, mean ± standard error of the percentage reduction of outward K^+ current by QUIN (100 μM) under control conditions and in the presence of ryanodine (data calculated at test membrane potential of +70 mV; holding potential, −50 mV). A, Ryanodine (20 μM; dissolved in ethanol) was included in the bathing solution. It can be seen that the effects of QUIN were markedly reduced. Control experiments contained 0.038% ethanol in the external solution. B, Ryanodine (10 μM) was added to the patch pipette solution. It can be seen that the intracellular application of ryanodine also significantly reduced the inhibition of outward current by QUIN. In this case, another group of control cells (0.019% ethanol in the pipette) were studied. *, $p < 0.005$.

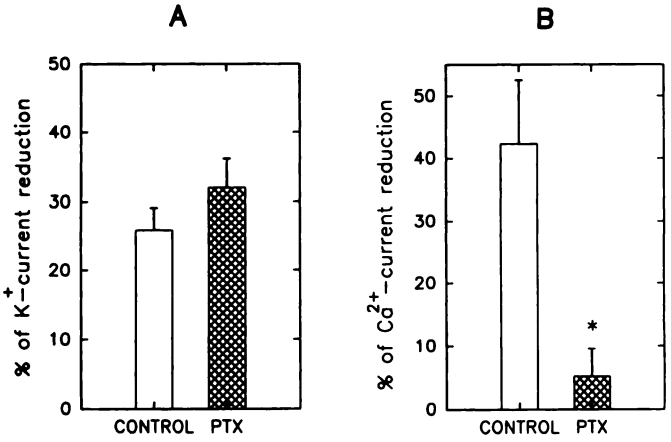

Fig. 9. Effect of pertussis toxin on the inhibition of two different ionic currents observed in transfected NG108–15 cells. A, *Bar graphs* demonstrating that pretreatment of the cells with pertussis toxin did not alter the percentage reduction of K^+ current produced by QUIN (100 μM). Data represent mean ± standard error of percentage reduction of the current observed at a test membrane potential of +70 mV (holding potential, −50 mV). Cells were incubated for 5 hr in normal culture solution containing pertussis toxin (1 μg/ml) (*PTX*) ($n = 8$) or not containing toxin (*CONTROL*) ($n = 8$). B, Demonstration that the pertussis toxin treatment used ADP-ribosylated G proteins in transfected NG108–15 cells. It can be seen that the inhibition of Ca^{2+} currents in these cells by DADLE was markedly reduced by pertussis toxin treatment. Cells were treated as described above (*PTX*, $n = 9$; *CONTROL*, $n = 7$). To study inward Ca^{2+} currents, the external solution contained (in mM) NaCl, 125; $BaCl_2$, 10.8; $CsCl_2$, 10.8; $MgCl_2$, 1.0; glucose, 10; and HEPES, 10; plus 2 μM tetrodotoxin, adjusted to pH 7.4 with Tris base. The patch pipette solution contained (in mM) $CsCl_2$, 120; $MgCl_2$, 3; Mg-ATP, 5; HEPES, 5; and EGTA, 10; adjusted to pH 7.4 with CsOH. To elicit Ca^{2+} currents, the cell membrane was held at −90 mV and stepped to membrane potentials between −100 and +40 mV for 300 msec. *, $p < 0.003$.

onstrate that a pertussis toxin-insensitive G protein was involved, we recorded from additional cells using patch electrodes that contained 100 μM GDPβS. This treatment had no effect on the native current studied but completely blocked the QUIN-induced augmentation of outward current (Fig. 10). Additional

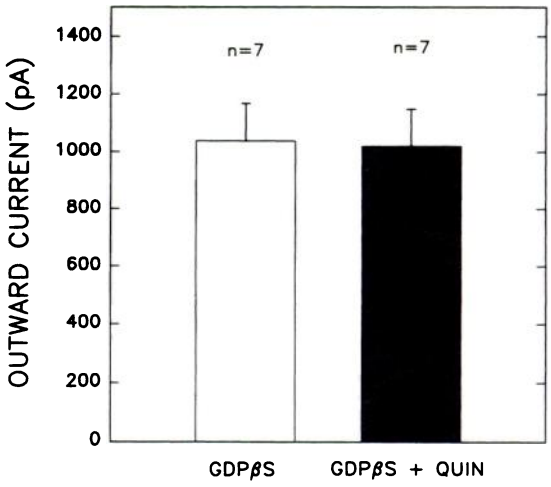

Fig. 10. Effects of intracellular application of GDPβS on the QUIN-induced decreases of outward current in transfected NG108–15 cells. When 100 μM GDPβS was included in the patch recording electrode, it was observed that the normal coupling of D_{2S} receptors to outward current was blocked. Control and QUIN data were collected with the same cells.

experiments were performed without the inclusion of cAMP in the recording pipette ($n = 10$). This had no effect on the currents observed in these cells or on the ability of QUIN application to inhibit outward current.

Discussion

The present observations demonstrate that, when D_{2S} receptors are transfected into NG108–15 cells, they inhibit the voltage-dependent K^+ current normally present. The pharmacological profile of this effect agreed with that typically ascribed to D_2 receptors, i.e., 1) DA and the D_2 receptor agonist QUIN produced similar, dose-dependent effects, 2) the selective D_1 agonist SKF 38393 was without effect on outward current, 3) the D_2 antagonist sulpiride blocked the effect of QUIN, whereas the D_1 antagonist SCH-23380 and the α-adrenergic receptor antagonist idazoxan did not alter this response, and 4) QUIN had no effect on outward current in untransfected cells. Moreover, our data indicated that the effect of D_{2S} activation on outward current was dependent upon the mobilization of intracellular Ca^{2+}. When the cells were loaded with 1 or 2 mM BAPTA, the QUIN-induced outward current inhibition was prevented. Two sources of Ca^{2+} can be used to increase the intracellular concentration of this ion, 1) release of Ca^{2+} from internal stores or 2) influx of extracellular Ca^{2+} through voltage-dependent Ca^{2+} channels opened during membrane depolarization. The latter process does not appear to be critical in the D_{2S} response observed, because neither reduction of the extracellular Ca^{2+} concentration nor blockade of Ca^{2+} channels by cobalt affected the QUIN-induced inhibition of K^+ current. In addition, it has been reported that DA and QUIN reduce the voltage-dependent Ca^{2+} current in the same D_{2S}-transfected cells used in this work (25). In support of mobilization of intracellular Ca^{2+} as a mechanism for the observed effects, thapsigargin (which increases intracellular Ca^{2+}) (26) was shown to mimic the effect of D_{2S} stimulation, whereas the blockade of intracellular Ca^{2+} channels with ryanodine completely abolished the D_{2S}-mediated reduction of outward current. The precise mechanism by which intracellular Ca^{2+} regulates voltage-dependent K^+ current in NG108–15 cells will require further study.

The modulation of outward K^+ current by D_{2S} receptor stimulation was not modified by pretreatment of the cells with pertussis toxin but was blocked by the inclusion of 100 μM GDPβS in the patch pipette. This observation indicates that coupling of the D_{2S} receptor to either G_i or G_o is not involved in the effects described above (27, 28). Although there are many reports showing that D_2 (3, 23) and D_{2S} receptors (17, 29, 30) are coupled to pertussis toxin-sensitive G proteins, pertussis toxin-insensitive D_2-mediated effects have also been observed. For example, Kanterman *et al.* (5) reported that the potentiation of arachidonic acid release induced by D_{2L} receptor stimulation was pertussis toxin resistant. In the last few years, pertussis toxin-insensitive G proteins have been purified from several tissues including brain. Interestingly, one of them, G_q, has been shown to stimulate phospholipase C and inositol trisphosphate production. In addition, inositol trisphosphate has been shown to induce Ca^{2+} release from intracellular stores in NG108–15 cells (31). This stimulation of phospholipase C has been shown to be involved in modulation of the actions of bradykinin on voltage-gated Ca^{2+} current in NG108–15 cells (32). The data presented here suggest that D_{2S} may be coupled to K^+ channels via a similar intracellular pathway. Further work will be necessary to test this possibility directly.

The present study also suggests a possible mechanism whereby D_2 receptor stimulation within the central nervous system may result in depolarization of the cell membrane. Although D_2 and D_{2S} receptors have generally been reported to decrease cell excitability by increasing K^+ current (7, 29, 33–37),[2] excitatory effects mediated by D_2 have also been observed (38–43). Only direct studies in the central nervous system will allow us to investigate whether mechanisms similar to those that we observed in NG108–15 cells transfected with the D_{2S} receptor may be underlying these *in vivo* excitatory actions of D_2 receptor stimulation.

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